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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : Zhou, Ming-Ming

SERIAL NO. : 09/784,553 EXAMINER : Lucas, Zachariah

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FOR : METHODS OF IDENTIFYING MODULATORS OF BROMODOMAINS

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Lois A. Snure
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Lois A. Snure 11/10/05
(Signature and Date)

I, Ming-Ming Zhou, Ph.D. as evidenced by my signature below, declare the following:

1. I am a professor in the Department of Physiology and Biophysics at Mt. Sinai School of Medicine and a Director in the Translational Chemical Biology Center at Mt. Sinai School of Medicine, having received my Ph.D. degree in Chemistry from Purdue University in 1993. After that, I served as a postdoctoral fellow at Abbott Laboratories in Chicago, Illinois.

2. My curriculum vitae is attached hereto as Exhibit A.

3. My principal area of research is Structural Biology, and among other positions I serve as reviewer in numerous scientific journals including Analytic Biochemistry, Biophysical Journal, EMBO Journal, European Journal of Biochemistry, FEBS Letters, GENE, JACS, Journal of Molecular Biology, Molecular Cell, Nature Structure Biology, Protein Science, Science and Structure. I also have served as a reviewer for funding agencies including the

American Cancer Society, the American Heart Association, the Israel Science Foundation, the National Science Foundation, the NIH and the European Commissions.

4. I am an inventor of subject matter claimed in the above-referenced patent application.

5. I am aware of many proteins containing a bromodomain that have been shown to interact with other proteins. Representative examples include the bromodomain of WSTF (Williams syndrome transcription factor) interacts with lysine-acetylated histones (Fujiki, R., et al., *Ligand-induced transrepression by VDR through association of WSTF with acetylated histones.* Embo J, 2005); the bromodomain of the transcriptional cofactor p300 binds to nucleosome (Ragvin, A., et al., *Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300.* J Mol Biol, 2004. **337**(4): p. 773-88); the bromodomain of CBP/p300 binds to acetylated MyoD (Polesskaya, A., et al., *Interaction between acetylated MyoD and the bromodomain of CBP and/or p300.* Mol Cell Biol, 2001. **21**(16): p. 5312-20); the bromodomain of NoRC (the SNF2h-containing chromatin-remodeling complex) interacts with K16-acetylated histone H4 (Zhou, Y. and I. Grummt, *The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing.* Curr Biol, 2005. **15**(15): p. 1434-8); the bromodomains of BDF1 and BDF2 bind to histone H4 (Matangkasombut, O., et al., *Bromodomain factor 1 corresponds to a missing piece of yeast TFIID.* Genes Dev, 2000. **14**(8): p. 951-62); the bromodomain of the WBSCR9 gene, encoding a novel transcriptional regulator, in the Williams-Beuren syndrome deletion at 7q11.23 (Peoples, R.J., et al., *Identification of the WBSCR9 gene, encoding a novel transcriptional regulator, in the Williams-Beuren syndrome deletion at 7q11.23.* Cytogenet Cell Genet, 1998. **82**(3-4): p. 238-46); the bromodomain-containing TIF1α: a possible link between KRAB zinc finger proteins and nuclear receptors (Le Douarin, B., et al., *TIF1alpha: a possible link between KRAB zinc finger proteins and nuclear receptors.* J Steroid Biochem Mol Biol, 1998. **65**(1-6): p. 43-50); the bromodomain of CBP interacts with human tumor suppressor p53 at acetylated lysine 372

(Mujtaba, S., et al., *Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation.* Mol Cell, 2004. **13**(2): p. 251-63).

6. I am aware of many proteins that have been shown to interact with a bromodomain of another protein. A major group of cellular proteins that interact with bromodomains are nucleosomal core histones H3, H4, H2A and H2B, each of which has multiple known lysine acetylation sites. In addition, other proteins including cellular proteins of p53 (Mujtaba, S., et al., *Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation.* Mol Cell, 2004. **13**(2): p. 251-63); NF-κB (Greene, W.C. and L.F. Chen, *Regulation of NF-kappaB action by reversible acetylation.* Novartis Found Symp, 2004. **259**: p. 208-17; discussion 218-25) and HIF1α (Chun, Y.S., et al., *Phorbol ester stimulates the nonhypoxic induction of a novel hypoxia-inducible factor 1alpha isoform: implications for tumor promotion.* Cancer Res, 2003. **63**(24): p. 8700-7) interact with a bromodomain of another protein.

7. I am aware of many instances where the consequences of interaction between a bromodomain and an acetyl lysine moiety have been described as regards biological activity. Examples of these include that the bromodomain containing 2 (Brd2) is expressed in distinct patterns during ovarian folliculogenesis independent of FSH or GDF9 action (Trousdale, R.K. and D.J. Wolgemuth, *Bromodomain containing 2 (Brd2) is expressed in distinct patterns during ovarian folliculogenesis independent of FSH or GDF9 action.* Mol Reprod Dev, 2004. **68**(3): p. 261-8); the bromodomain of the MLL-CBP fusion protein is required for generating a myelodysplastic-like syndrome that evolves into myeloid leukemia (Lavau, C., et al., *Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia.* EMBO J., 2000. **19**: p. 4655-4664); the bromodomain-containing histone H3 acetylase dGcn5 is a key player in Drosophila melanogaster metamorphosis(Carre, C., et al., *The histone H3 acetylase dGcn5 is a key player in Drosophila melanogaster metamorphosis.* Mol Cell Biol, 2005. **25**(18): p. 8228-38); the bromodomain protein Brd4 is a positive regulatory

component of P-TEFb and stimulates RNA polymerase II-dependent transcription (Jang, M.K., et al., *The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription.* Mol Cell, 2005. **19**(4): p. 523-34); the PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing (Jang, M.K., et al., *The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription.* Mol Cell, 2005. **19**(4): p. 523-34); the bromodomain-containing protein Bdf1p acts as a phenotypic and transcriptional multicopy suppressor of YAF9 deletion in yeast (Bianchi, M.M., et al., *The bromodomain-containing protein Bdf1p acts as a phenotypic and transcriptional multicopy suppressor of YAF9 deletion in yeast.* Mol Microbiol, 2004. **53**(3): p. 953-68); Bdf1 bromodomains' interactions with acetylated H4 tails help anchor the transcriptional protein complex TFIID to the promoter during the initial stages of transcription activation (Martinez-Campa, C., et al., *Precise nucleosome positioning and the TATA box dictate requirements for the histone H4 tail and the bromodomain factor Bdf1.* Mol Cell, 2004. **15**(1): p. 69-81); the CBP bromodomain and nucleosome targets are required for Zta-directed nucleosome acetylation and transcription activation (Deng, Z., et al., *The CBP bromodomain and nucleosome targeting are required for Zta-directed nucleosome acetylation and transcription activation.* Mol Cell Biol, 2003. **23**(8): p. 2633-44); the bromodomains anchor chromatin-modifying complexes to promoter nucleosomes (Hassan, A.H., et al., *Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes.* Cell, 2002. **111**: p. 369-379); the bromodomain mediates transcriptional intermediary factor 1alpha and nucleosome interactions (Remboutsika, E., et al., *The bromodomain mediates transcriptional intermediary factor 1alpha -nucleosome interactions.* J Biol Chem, 2002. **277**(52): p. 50318-25).

8. I have reviewed Zeng *et al., FEBS Letters* 513:124-128 cited by the Examiner. As reported in the specification of the above-referenced patent application, some bromodomains may not bind to the free amino acid acetyl-lysine alone. This may be due to the charged amino and/or carboxyl groups of the amino acid lysine that are adjacent to its acetyl moiety. However,

bromodomains do in fact interact with and bind to an acetyl-lysine residue when it is presented in a polypeptide sequence such as those in proteins. In latter cases, these charged groups are naturalized due to polypeptide connectivity. Hence, the acetyl lysine may be necessary for bromodomains to bind to a particular portion of a protein.

9. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, or any patent issuing thereon.

Submitted by

Ming-Ming Zhou, Ph.D.

Date Signed: November 10, 2005